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Final Report: Acquisition of a Raman Microscope for Interdisciplinary Research and Education

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**6. AUTHORS**
Kai Shen, Christopher Hintz, Hua Zhao, Paramasivam Sivapatham, Karla Sue Marriott

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAMES AND ADDRESSES**
Savannah State University
3219 College Street

Savannah, GA 31404 -5254

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U.S. Army Research Office
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The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

**14. ABSTRACT**
Supported by this DoD/ARO instrument grant, Savannah State University (SSU) acquired, installed, and tested a state-of-the-art Horiba LabRAM HR Evolution Raman Microscope system. The instrument has been employed in both research and education activities and greatly enhanced the University's research and teaching capabilities. Specifically, the major achievements during the reporting period include: (1) The Raman microscope was successfully installed and tested in December 2015. (2) Dr. Christopher Hintz, the system administrator of the Raman microscope, attended a comprehensive training at Horiba's headquarter to better manage the instrument. (2)

**15. SUBJECT TERMS**
Raman spectroscopy, Interdisciplinary research and education, Historically Black Colleges and Universities (HBCU)

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 15. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | Kai Shen |
| UU | UU | UU | UU | | 19b. TELEPHONE NUMBER 912-358-4437 |

## Report Title

Final Report: Acquisition of a Raman Microscope for Interdisciplinary Research and Education


## ABSTRACT

Supported by this DoD/ARO instrument grant, Savannah State University (SSU) acquired, installed, and tested a state-of-the-art Horiba LabRAM HR Evolution Raman Microscope system. The instrument has been employed in both research and education activities and greatly enhanced the University's research and teaching capabilities. Specifically, the major achievements during the reporting period include: (1) The Raman microscope was successfully installed and tested in December 2015. (2) Dr. Christopher Hintz, the system administrator of the Raman microscope, attended a comprehensive training at Horiba's headquarter to better manage the instrument. (3) The PI has employed the instrument in a DoD sponsored research project to study protein conformation changes. (4) The instrument has been introduced to more than 60 undergraduate students who attended the following upper level undergraduate courses: Biochemistry, Principles of Forensic Science, Principles of Forensic Science Lab. (5) Two SSU undergraduate students and two DoD High School Apprenticeship Program (HSAP) summer interns were trained to utilize the Raman microscope in their research projects. (6) Two local middle school at the nationally-recognized STEM Academey at Bartlett were introduced and used data collected on the instrument for their science fair projects. (7) The instrument was also introduced to potential industrial and academic users including J.C. Bamford Excavators Limited and Armstrong State University.


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## Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

### (a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

**TOTAL:**

**Number of Papers published in peer-reviewed journals:**

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### (b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

**TOTAL:**

**Number of Papers published in non peer-reviewed journals:**

---

### (c) Presentations

Sakura McLaughlin, Terrence Cumby, Karla Sue Marriot, and Kai Shen "Structural Changes of Sigma-1 Receptor upon Binding to Its Agonist", SSU Research Conference & RIMI Symposium, 2016

**Number of Presentations:** 1.00

## Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

**TOTAL:**

**Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

## Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

**TOTAL:**

**Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):**

## (d) Manuscripts

Received Paper

**TOTAL:**

**Number of Manuscripts:**

## Books

Received Book

**TOTAL:**

| Received | Book Chapter |
|---|---|

**TOTAL:**

## Patents Submitted

## Patents Awarded

## Awards

LaTanya Downer, an undergraduate student involved in a project using the instrument, received the University of Kansas Research Experiences for Undergraduates (REU) 2016 Scholarship

## Graduate Students

| NAME | PERCENT_SUPPORTED |
|---|---|
| **FTE Equivalent:** | |
| **Total Number:** | |

## Names of Post Doctorates

| NAME | PERCENT_SUPPORTED |
|---|---|
| **FTE Equivalent:** | |
| **Total Number:** | |

## Names of Faculty Supported

| NAME | PERCENT_SUPPORTED | National Academy Member |
|---|---|---|
| Kai Shen | 0.00 | |
| Christopher Hintz | 0.00 | No |
| Karla Sue Marriott | 0.00 | |
| Hua Zhao | 0.00 | |
| Paramasivam Sivapatham | 0.00 | |
| **FTE Equivalent:** | **0.00** | |
| **Total Number:** | **5** | |

## Names of Under Graduate students supported

| NAME | PERCENT_SUPPORTED | Discipline |
|---|---|---|
| Tiffany Villanueva | 0.00 | Forensic Science |
| LaTanya Downer | 0.00 | Chemistry |
| **FTE Equivalent:** | **0.00** | |
| **Total Number:** | **2** | |

## Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: ..... 2.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 2.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 2.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 2.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense ..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 1.00

## Names of Personnel receiving masters degrees

| NAME |
|---|
| **Total Number:** |

## Names of personnel receiving PHDs

| NAME |
|---|
| **Total Number:** |

## Names of other research staff

| NAME | PERCENT_SUPPORTED |
|---|---|
| **FTE Equivalent:** | |
| **Total Number:** | |

## Sub Contractors (DD882)

## Inventions (DD882)

# Scientific Progress

(1) Foreword

Raman spectroscopy is a powerful tool to characterize material properties such as protein and DNA conformations, chemical compositions of marine and soil samples. The Raman microscope acquired with this DoD/ARO grant has multiple excitation wavelengths (244, 785, and 532 nm), high spectral resolution (1 cm-1), high spatial resolution (< 1 ◡m), and sample temperature control stage. The unique material information derived from this state-of-the-art Raman microscope enables the faculty at Savannah State University, a historically Black University (HBCU), to solve challenging research questions. Besides, the instrument has been introduced in our undergraduate and graduate courses, and our students have learned applications of Raman spectroscopy and gained hands-on experience with the Raman microscope.

(2) Statement of the problem studied

Research projects

One goal of this instrument acquisition is to enable faculty members at Savannah State University, who are from various disciplines, to address their research questions. During the reporting period, a total of five research projects from different disciplines have employed or are employing the instrument to collect high resolution Raman spectra for their studies.

The first project is to investigate structural changes of sigma-1 receptor upon cholesterol or agonist binding. Sigma-1 receptor is a cholesterol-binding membrane protein and has shown neuroprotective properties when binding to its agonists in Alzheimer' s disease. Sigma-1 receptor is believed to regulate cholesterol homeostasis and may slow the progression of Alzheimer's disease. However, the underlying mechanism of such cholesterol regulations remains to be elucidated. With the Raman spectrometer equipped with a UV laser (244 nm), the two core users Drs. Kai Shen and Karla Sue Marriott are focusing on locating structural features of sigma-1 receptor that are critical for cholesterol and agonist binding.

The second project is to investigate the effect of phospholipid interaction and phosphorylation on structural changes of focal adhesion protein vinculin. The goal of this project is to use Raman spectroscopy to monitor changes in conformation of vinculin when bound to phosphoinositol 4,5 bisphosphate (PIP2) and subjected to phosphorylation. Vinculin and its muscle splice variant, metavinculin, regulate cell adhesion and cytoskeleton remodeling in response to environmental cues and are vital for cell survival, development, and migration. Specifically, competitive binding of PIP2 toward vinculin tail region regulates force transmission, actin cytoskeleton remodeling, and cell motility. Similarly, phosphorylation of vinculin Y1065 is essential for force generation and cell mechanotransduction.

The third project, led by Dr. Christopher Hintz, aims to characterize microstructure variation in calcite biominerals. Understanding biomineralization mechanisms and their inherent microheterogeneity is necessary to improve our abilities to develop past ocean/climate records. Shell heterogeneities have been observed by a number of micro analytical methods in an attempt to improve our understanding of the minerals and the processes by which they are made. However, to date, all of these techniques are destructive to the sample, and in some cases alter the sample (and its analytical results) during the analysis. Dr. Hintz's lab is developing several non-destructive micro-Raman (μR) techniques and uses them to investigate foraminiferal test (shell) micro-heterogeneities.

The fourth project, developed after acquisition of the μR instrumentation, also led by Dr. Hintz and assisted by Dr. Sue Ebanks, investigates micro plastics found in the local environment, positively identifying the microplastic materials. The methods involved are still under development but show promise through two K-12 student and a single undergraduate project.

The fifth project, led by Dr. Hua Zhao, probes DNA-ionic solvent interactions using Raman spectroscopy. This project is to utilize Raman spectroscopy along with other methods to understand the interactions between DNA and ionic solvents (e.g. ionic liquids and deep eutectic solvents), aiming to guide the DNA-based asymmetric catalysis. DNA has been used as a chiral scaffold for metal complexes to produce so called 'DNA-based hybrid catalysts'. These new hybrid catalysts are very promising for asymmetric synthesis and have shown high activities and enantioselectivities in several reactions including the Michael addition, Diels-Alder reaction, and Friedel-Crafts. Different from aqueous solutions, using ionic liquid has shown high compatibility with biocatalysts and can maintain certain triplex and G-quadruplex structures of DNA. Raman spectroscopy is used to understand DNA behaviors in ionic solvents, which will enable us to rationally design ionic liquids and DES systems for DNA-based asymmetric synthesis.

The sixth project is led by Dr. Paramasivam Sivapatham. The goal of this project is to understand the reactions phosphorus fertilizer in soils and transformations of various phosphorus species on qualitative basis in soils and other environmental systems. Consumption of P fertilizers has been continuously increasing worldwide. Decreasing phosphate resources and increasing price of phosphate rock along with increasing consumption of P fertilizers worldwide for crop production lead to 'A Potential Phosphorus Crisis'. Understanding of phosphorus fertilizer reactions and speciation in soils will potentially help improve P use efficiency and minimize P fertilizer use for sustained crop production. Dr. Sivapatham's group uses Raman spectroscopy to gain qualitative knowledge of various phosphorus species and their associations in the presence of major soil cations such as Ca, Mg, Fe and Al at different growth stages of potato crop over the period of growing season following the application of phosphorus fertilizer application.

Education goals

The Raman microscope is also used in teaching activities. Besides the research students involved in the aforementioned projects, SSU students enrolled in upper level undergraduate and graduate courses in chemistry, forensic science, and marine science have opportunities to learn principles of Raman spectroscopy and have access to the instrument. Through collaboration, local middle and high school teachers and students also have opportunities to work with the instrument.

(3) Summary of the most important results/progress

During the reporting period, we have acquired, installed, and tested the instrument. Several research and education activities

using the instrument have been carried out as proposed. The major results are summarized below:
For project 1, Drs. Shen and Marriott have prepared sigma-1 receptor and variants for Raman resonance assignments. Since sigma-1 receptor is a transmembrane protein, the researchers also successfully incorporated the protein into micelles and nanodiscs for characterizing changes in Raman spectra when adding agonists and cholesterol.
For Project 2, Drs. Shen and Marriott have purified vinculin tail variants including W1058, W1064, Y1065. They are using these mutants to assign Raman resonances. After assignment, they will probe structural changes of vinculin tail in the presence of phosphoinositol 4,5 bisphosphate (PIP2).
For Project 3, Dr. Hintz has carried out preliminary tests on the collected marine samples. Commitments to graduate students on other research projects have limited progress on this project.
For Project 4, Drs. Hintz and Ebanks have worked with multiple K-12 and undergraduate students analyzing microplastics collected from the local estuarine environment. Through these studies, pristine and polluted sites highly correlate with population density in the nearby islands.
For Project 5, Dr. Zhao has prepared a series of DNA based catalysts and will use Raman spectroscopy to monitor resonance shifts of ionic liquid in the absence and presence of DNA based catalysts.
For Project 6, Dr. Sivapatham has collected soil samples and will use Raman spectroscopy to characterize changes of phosphorus species in a series of samples.
We have also made progress in achieving our education goal. During the reporting period, the PI introduced the instruments to students who attended biochemistry, Principle of Forensic Science and Principle of Forensic Science Laboratory courses. More than 60 students have learned the basic principles and applications of Raman spectroscopy in biological science and forensic science. Two 2016 DoD High School Apprenticeship Program (HSAP) scholars hosted in the PI's lab also learned basics of Raman spectroscopy and gained hands-on experience using the Raman spectrometer. Two middle school students at the national-award-winning STEM Academy at Bartlet utilized data collected by the Raman instrument for their science project investigating microplastics in the environment.

## Technology Transfer

N/A

# Scientific Progress (W911NF-15-1-0037)

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